

<b>REPORT DOCUMENTATION PAGE</b>			Form Approved OMB NO. 0704-0188		
<p>The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA, 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.</p> <p>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</p>					
1. REPORT DATE (DD-MM-YYYY) 20-12-2015		2. REPORT TYPE Final Report		3. DATES COVERED (From - To) 1-Oct-2012 - 30-Sep-2015	
4. TITLE AND SUBTITLE Final Report: Proteins as Supramolecular Building blocks for Responsive Materials and Nanodevices			5a. CONTRACT NUMBER W911NF-12-1-0554		
			5b. GRANT NUMBER		
			5c. PROGRAM ELEMENT NUMBER 611102		
6. AUTHORS Gerrard, JA; Mabbutt, BC; Steel, PJ			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAMES AND ADDRESSES University of Canterbury 20 Kirkwood Ave Ilam			8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES) U.S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211			10. SPONSOR/MONITOR'S ACRONYM(S) ARO		
			11. SPONSOR/MONITOR'S REPORT NUMBER(S) 61663-CH.9		
12. DISTRIBUTION AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.					
14. ABSTRACT Our research programme exploring the use of two toroidal proteins as tectons for nanotechnological applications has been successfully completed.  In addition to the work funded by this programme, significant synergies were gained with a parallel programme funded by the New Zealand government, which has incorporated the proteins and peptides designed in this programme into useful materials and devices.					
15. SUBJECT TERMS protein, supramolecular, nanotechnology, self-assembly					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON Juliet Gerrard
a. REPORT UU	b. ABSTRACT UU	c. THIS PAGE UU			19b. TELEPHONE NUMBER +64-364-2987



## Report Title

Final Report: Proteins as Supramolecular Building blocks for Responsive Materials and Nanodevices

### ABSTRACT

Our research programme exploring the use of two toroidal proteins as tectons for nanotechnological applications has been successfully completed.

In addition to the work funded by this programme, significant synergies were gained with a parallel programme funded by the New Zealand government, which has incorporated the proteins and peptides designed in this programme into useful materials and devices.

---

**Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:**

**(a) Papers published in peer-reviewed journals (N/A for none)**

<u>Received</u>	<u>Paper</u>
08/11/2013	1.00 Céline Valéry, Rishi Pandey, Juliet A. Gerrard. Protein beta-interfaces as a generic source of native peptide tectons, Chemical Communications, (12 2013): 2825. doi: 10.1039/c3cc39052g
08/24/2014	2.00 Amy J. Phillips, Jacob Littlejohn, N. Amy Yewdall, Tong Zhu, Céline Valéry, F. Grant Pearce, Alok K. Mitra, Mazdak Radjainia, Juliet A. Gerrard. Peroxiredoxin is a Versatile Self-Assembling Tecton for Protein Nanotechnology, Biomacromolecules, (05 2014): 0. doi: 10.1021/bm500261u
12/20/2015	5.00 Jenny Malmström, Akshita Wason, Fergus Roache, N. Amy Yewdall, Mazdak Radjainia, Shanghai Wei, Michael J. Higgins, David E. Williams, Juliet A. Gerrard, Jadranka Travas-Sejdic. Protein nanorings organized by poly(styrene-block-ethylene oxide) self-assembled thin films, Nanoscale, (11 2015): 0. doi: 10.1039/C5NR05476A
12/20/2015	6.00 Galen L. Eakins, Rishi Pandey, Jonathan P. Wojciechowski, Han Yue Zheng, James E. A. Webb, Céline Valéry, Pall Thordarson, Natalie O. V. Plank, Juliet A. Gerrard, Justin M. Hodgkiss. Functional Organic Semiconductors Assembled via Natural Aggregating Peptides, Advanced Functional Materials, (09 2015): 0. doi: 10.1002/adfm.201502255
12/20/2015	7.00 Mazdak Radjainia, Hariprasad Venugopal, Ambroise Desfosses, Amy J. Phillips, N. Amy Yewdall, Mark B. Hampton, Juliet A. Gerrard, Alok K. Mitra. Cryo-Electron Microscopy Structure of Human Peroxiredoxin-3 Filament Reveals the Assembly of a Putative Chaperone, Structure, (05 2015): 0. doi: 10.1016/j.str.2015.03.019
<b>TOTAL:</b>	<b>5</b>

Number of Papers published in peer-reviewed journals:

---

**(b) Papers published in non-peer-reviewed journals (N/A for none)**

Received      Paper

**TOTAL:**

Number of Papers published in non peer-reviewed journals:

---

**(c) Presentations**

Number of Presentations: 3.00

---

**Non Peer-Reviewed Conference Proceeding publications (other than abstracts):**

Received      Paper

**TOTAL:**

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

---

**Peer-Reviewed Conference Proceeding publications (other than abstracts):**

Received      Paper

**TOTAL:**

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received      Paper

TOTAL:

Number of Manuscripts:

Books

Received      Book

TOTAL:

Received      Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Juliet Gerrard: Jefferey Lectureship, University of New South Wales

Six student poster prizes - see highlights  
Two student talk prozes - see highlights

---

### Graduate Students

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	Discipline
Amy Yewdall	1.00	
New Entry	0.00	
<b>FTE Equivalent:</b>	<b>1.00</b>	
<b>Total Number:</b>	<b>2</b>	

---

### Names of Post Doctorates

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
<b>FTE Equivalent:</b>	
<b>Total Number:</b>	

---

### Names of Faculty Supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
<b>FTE Equivalent:</b>	
<b>Total Number:</b>	

---

### Names of Under Graduate students supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
<b>FTE Equivalent:</b>	
<b>Total Number:</b>	

---

### Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: ..... 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense ..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: ..... 0.00

---

### Names of Personnel receiving masters degrees

<u>NAME</u>
<b>Total Number:</b>

---

**Names of personnel receiving PhDs**

<u>NAME</u>
-------------

<b>Total Number:</b>
----------------------

---

**Names of other research staff**

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
-------------	--------------------------

Bhumika Shah	1.00
--------------	------

<b>FTE Equivalent:</b>	<b>1.00</b>
------------------------	-------------

<b>Total Number:</b>	<b>1</b>
----------------------	----------

---

**Sub Contractors (DD882)**

**Inventions (DD882)**

**Scientific Progress**

See attachment

**Technology Transfer**

See attachment for connection to other research programmes

## 1. Forward and executive summary

**Background:** Our long term objective is to create and customise new components appropriate to bio-responsive materials and devices. Proteins self-assemble in water-based environments, a property that we are exploiting in the fabrication of novel supramolecular architectures. In this programme, we have used two complementary model systems that spontaneously form ring structures according to their inherent biology:

1. The Lsm proteins create ring assemblies to gather up RNA and protein partners within their scaffold architecture.
2. The peroxiredoxin (Prx) proteins also self assemble into rings, once triggered by a redox switch, and then into intriguing superstructures.

In each case, our understanding of external factors determining self-association has allowed us to drive the two systems into stable organised nanoscale structures. We have enhanced and extended these structural forms and added motifs by attaching other moieties to add functionality. An ability to exquisitely control the architecture and assembly of the Lsm and Prx in solution and at surfaces, as well as to tune their molecular binding events, has supplied the field with two new, versatile, building blocks (tectons) with which to build intelligent materials. These are being used in related programmes, for example to add extra functional scope to materials ordered at the nanoscale. Ultimately, these will have capacity to sense and respond to specific stimuli, including those of military relevance.

### Highlights for the programme:

- Publication of a paper describing the first 3D structures of a toroid, double toroid, and nanotubes of hPrx3: Phillips, A.J., Littlejohn, J., Yewdall, N.A., Zhu, T., Valéry, C., Pearce, F.G., Mitra, A.K., Radjainia, M., Gerrard, J.A. 2014: Peroxiredoxin is a versatile self-assembling tecton for protein nanotechnology, *Biomacromolecules*, 15: 1871-1881.
- Award of a prize for best poster talk to Amy Phillips at the MacDiarmid Institute Symposium, November 2013.
- Lorne poster prize for Amy Yewdall: Yewdall N.A., Valery C., Sasso L., Pearce F.G., Gerrard J.A. (2014) The functional impact of manipulating peroxiredoxin quaternary structure. 39<sup>th</sup> Lorne Conference on Protein Structure and Function; awarded student poster prize.
- Lorne poster prize (2014, 2015) for Francesca Manea: Manea, F., Pearce, F.G. Mabbutt, B.C. (2014) Engineering bio building blocks: the architecture and control of synthetic ring-forming proteins; AND Manea F., Shah B. & Mabbutt, B.C (2015) Building protein nanomaterials: engineering with synthetic Lsm ring tectons, 39<sup>th</sup> and 40<sup>th</sup> Lorne Conference on Protein Structure and Function; student poster awards.
- Queenstown Poster prize for Amy Yewdall: Yewdall N. A., Pandey R., Phillips A. J., Hampton M., Pearce F. G., Gerrard J. A. (2014) Functional consequences of structural manipulation of Human Peroxiredoxin 3.



- Student talk prize for Amy Yewdall at the School of Biological Sciences Annual Conference, University of Canterbury.
- Student talk prize for Amy Yewdall at the College of Science PhD in 3 competition, University of Canterbury.
- Amy Phillips has successfully completed her PhD thesis: 'Peroxiredoxins as self assembling building blocks for nanotechnology'
- Akshita Wason has successfully completed PhD thesis: 'Investigation of Lsm Proteins as Scaffolds in Bionanotechnology'
- Francesca Manea has submitted her PhD thesis: 'Engineering synthetic Lsm rings for applications in nanotechnology'
- Formation of stacks of both Lsm and Prx proteins and increased understanding of how to control the stack formation.
- Publication of an edited volume in the Humana Methods in Molecular Biology Series: Gerrard, J.A. (Ed.) 2013: Protein Nanotechnology – Protocols, Instrumentation, Applications. Second Edition. Edited by Gerrard with contributions from Gerrard and Mabbutt.
- Publication: Valéry, C., Pandey, R. and Gerrard J.A. 2013: Protein  $\beta$ -interfaces as a generic source of native peptide tectons. *Chemical Communications* 49: 2825-2827.
- Award of first prize in the poster competition to Amy Phillips, at the British Biochemical Society meeting, for her poster: Phillips, A.J., Valery, C., Radjainia, M., Mitra, A., Lott, S., Gerrard, J.A. 2013: Responsive scaffolds from protein nano-donuts. Protein Engineering: new approaches and applications, University of Chester, UK, 10-12 April 2013.
- Publication of a high resolution cryo-EM structure of the peroxiredoxin filament: Radjainia M, Venugopal H, Desfosses A, Phillips AJ, Yewdall NA, Hampton MB, Gerrard JA, Mitra AK. 2015: Cryo-electron microscopy structure of human peroxiredoxin-3 filament reveals the assembly of a putative chaperone. *Structure* 23(5):912-920.
- Publication of a paper from a related research programme, incorporating the Lsm tecton into a block co-polymer (includes cover image): Malmström J, Wason A, Roache F, Yewdall NA, Radjainia M, Wei S, Higgins MJ, Williams DE, Gerrard JA, Trivas-Sejdic J. Protein nanorings organized by poly(styrene-block-ethylene oxide) self-assembled thin films. *Nanoscale*, published 26 Oct 2015.
- Publication of a paper from a related research programme, showing the utility of our self-assembling peptide tectons in advanced materials: Eakins GL, Pandey R, Wojciechowski JP, Zheng HY, Webb JEA, Valéry C, Thordarson P, Plank NOV, Gerrard JA, Hodgkiss JM. 2015: Functional Organic Semiconductors Assembled via Natural Aggregating Peptides *Advanced Functional Materials* 25(35):5640-5649.
- Successful incorporation of an artificial amino acid into the Prx tecton which makes click chemistry accessible in this system.

## 2. Table of contents

1. Forward, summary, highlights	1
2. Table of contents	3
3. Personnel	4
4. List of figures and tables	5
5. Statement of the problem studied	6
6. Model systems chosen	7
7. Summary of the most important results, by objective	8
Y1.1 Investigation of Lsm ring forms engineered with altered diameter and pores	
Y1.2 Physical characterisation of morphology and control of stacked forms of Lsm	
Y1.3 Generation of Cys and other derivative forms of Lsm rings	
Y1.4 Characterisation of assembly properties of hPrx3 and hPrx3S78A with and without His-tag at a range of pHs	
Y1.5 Characterisation of B-peptide interaction with toroidal stacks of hPrx3 and Lsm (and mutants)	
Y1.6 Characterisation of assembly properties of hPrx3 and appropriate mutants using tags, metal ions and redox conditions	
Y2.1 Physical characterisation of morphology and control conditions for Lsm polyprotein polymers	
Y2.2 Physical characterisation of morphology and control of stacked forms of Lsm	
Y2.3 Physical characterisation and exploration of solvent conditions for Lsm arrays	
Y2.4 Functionalisation and assembly of Prx derivatised with fluorophores	
Y2.5 Engineering hPrx3 toroids of increased pore size and analysing the influence on their propensity to assemble into stacks	
Y2.6 Testing of appropriate toroidal structures in metal-templated assembly	
Y3.1 Generation of multifunctional Lsm rings utilising polyproteins	
Y3.2 Derivatisation of Lsm in array or tubule forms	
Y3.3 Crystallography of Lsm forms	
Y3.4 Functionalisation and assembly of Prx derivatised with antimicrobial peptides	
Y3.5 Cloning, expression and characterization of assembly properties of appropriate thermophilic Prx and Lsm, and mutants, building on the results of Years 1 and 2	
Y3.6 Testing of appropriate toroidal structures as surface coatings	
8. Conclusions	16
9. Bibliography	17

### 3. Personnel

The ARO funding supplemented funding from a variety of other sources in NZ and Australia, which supported the following personnel. Collaborative meetings were held regularly.

- Prof Juliet Gerrard, programme leader, time funded by University of Canterbury and Callaghan Innovation. **Note that from October 1<sup>st</sup> Juliet is took up a new Professorial role at the University of Auckland. This did not impact on her ability to lead the programme.**
- A/Prof Bridget Mabbutt, PI, time funded by Macquarie University.
- Prof Peter Steel, PI, time funded by University of Canterbury.
- Dr Grant Pearce, AI, 90% funded by Biomolecular Interaction Centre (BIC) at the University of Canterbury; 10% funded by this grant from January 2014 (replaces Celine Valery).
- Dr Valery left the University in Dec 2013 to take up a role at RMIT in Melbourne; we continue to collaborate.
- Francesca Manea, completed PhD thesis on Lsm in Mabbutt lab, August 2015, funded by Macquarie University.
- Jacob Littlejohn, completed MSc in Gerrard lab on peroxiredoxins, funded by UC; took up Macquarie PhD scholarship in Mabbutt lab, commencing March 2013; discontinued 2015 for personal reasons.
- Amy Phillips, completed PhD in Gerrard lab January 2014, on peroxiredoxins, funded by BIC and MacDiarmid Institute.
- Akshita Wason, completed PhD on Lsm proteins, August 2014, co-supervised by Gerrard and Mabbutt, funded by the MacDiarmid Institute
- Amy Yewdall, completing PhD in Gerrard lab on peroxiredoxins, funded by this grant. Amy will spend time in Auckland in 2015, facilitating access to high resolution cryo-EM.
- Rishi Pandey, completing PhD on self-assembling peptides in Gerrard lab, co-supervised by Valery, funded by BIC and AgResearch and the Riddet Institute; minor contribution to this programme.
- Helen Ashmead, completing PhD in related programme funded by Callaghan Innovation.
- Postdoc at Macquarie University, Dr Bhumika Shah, commenced December 2013, with expertise in protein crystallisation and crystallography.
- Dominic Logel, Masters Research student for 2016, Macquarie University, under supervision of A/Prof. Mabbutt.

#### 4. List of figures and tables

- Figure 1: Examples of Lsm rings comprising 6,7 and 8 protomer units. These distinct arrangements are naturally formed by Lsm proteins Hfq, MtLsm<sup>2</sup> and γLsm3, respectively.
- Figure 2: Self assembly of the peroxiredoxins. From left to right: dimer, decamer, catenane, dodecahedron, nanotubule.
- Figure 3: Figure 3: artists impression of Lsm proteins organised within a block co-polymer, as featured on the inside front cover of Nanoscale, November 2015.
- Figure 4: LC-MS data for unnatural amino acid (UAA) protein before and after click chemistry with DBCO-Cy5 fluorophore. The molecular mass is for monomeric protein, as seen in the cartoon representation of the crystal structure. Absorbance at 280 nm shows protein peak eluting from reversed-phase HPLC column, clicked protein having a slower retention time than the un-clicked protein.
- Figure 5: Controlling the size of hPrx3 stacked assemblies. Constructs with 6,4, or 2 histidines at the N-terminus form populations of different lengths in both solution, measured here by AUC and by TEM (inset) at different pHs.
- Figure 6: DSF profile for *apo* Lsm[2+3]<sub>4</sub> (cyan) and Lsm[2+3]<sub>4</sub> with U<sub>2</sub>GCU<sub>4</sub> RNA (orange) (2 mg/mL) exposed to heating at 1 ° C/min with 200 x SYPRO Orange solution. Derivative trace is upper right.
- Figure 7: Engineering and characterisation of Lsm polyprotein Cys-linked cluster. SEC at pH 8.0 reveals a high MW organisation that can be individually isolated. Cluster morphology of approximately 12 nm is seen using EM (2.2 % uranyl acetate stain, 130 000x magnification). The reversibility of the system is illustrated with the addition of TCEP to reduce disulfide bonds, resulting in a large single ring population. Removal of the reducing agent leads to the generation of the protein cluster.
- Figure 8: Physical properties of 4\*Ni-Lsm[4+1]<sub>4</sub>. SEC illustrates the concentration dependant relationship between protein and titrated metal. Clusters of 4\*Ni-Lsm[4+1]<sub>4</sub> visualised by EM, subsequently dissolve to single ring tectons with addition of EDTA.
- Figure 9: TEM visualization of 2Cu-Lsm3<sub>8</sub> Electron micrograph at pH 8.0, negatively stained (uranyl acetate) at 130 000x. Individual selected particles display a pore. Selected ring particles observed following addition of EDTA are also shown.
- Figure 10: Crystals of Lsm [4+1] single ring are shown on the left in 0.2 M magnesium acetate, 20 % PEG 3350. The diffraction pattern at 3.2 Å is shown on the right.
- Figure 11: Crystalline material & diffraction collection LsmαR65P. (A-D) sparse matrix screens for LsmαR65P with and without U<sub>4</sub>CGU<sub>4</sub>. (E) Diffraction obtained from crystal depicted in D)
- Figure 12: Preliminary AFM data showing hPrx3 ordered on a surface containing gold nanodots at defined spacing.

## 5. Statement of the problem studied

We aimed to use protein engineering, in combination with *e.g.* metal- and peptide-templated processes, to exquisitely control supramolecular assembly of two new protein building blocks at the nanometre scale, with a long term view to fabricate them as responsive materials and nanodevices.

Biology is an unparalleled source of nanostructures that are rich in form, highly varied in function and have an impressive ability to respond to specific stimuli. The inherent potential of this source of molecular scaffolds for the generation of responsive materials and nanodevices has been repeatedly noted over the last ten years, with pioneer case studies emerging in the field (Hedde *et al.*, 2007; Ballister *et al.*, 2008; Behrens *et al.*, 2009; Ulijn & Woolfson, 2010). In the same decade, supramolecular chemists have made huge strides in controlled self-assembly of designer molecules with a view to creating functional entities (Schalley *et al.*, 2004; Steel, 2005; Saalfrank *et al.*, 2008; Ling *et al.*, 2009; Zayed *et al.*, 2010). However, this excitement is tempered by difficulties in generating species of adequate scale for engineering truly practical and/or functional materials: well characterised supramolecular systems typically employ components of 1-2 nm, whereas nanoscale devices demand 10-100 nm dimensions. The union of supramolecular chemistry and protein science is thus very timely, and provides an opportunity to combine supramolecular elements with nanoscale biomolecules (Whitesides, 2003) not only for the study of biological phenomena (Uhlenheuer *et al.*, 2010) but also for the generation of useful materials.

For progress in protein supramolecular assembly to match that in the DNA realm (Bath *et al.*, 2007; Zheng, *et al.*, 2009), a range of building blocks of defined architectures and simply controlled assembly events is urgently required. This proposal seeks to fill this gap by developing self-assembling toroidal structures as supramolecular building blocks. We are exploiting the capacity of proteins for self-assembly in an aqueous environment to fabricate novel supramolecular architectures. We are utilising two protein systems as examples, selected for their inherent ability to self-assemble into a variety of forms. Both proteins have a propensity to form large ring structures, yet differ in their biochemical features and assembly properties and thence utility for applications *in vitro*. The structures, within the range ~20-100 nm, are relatively large compared to structures used up to now in physical nanoscience and supramolecular chemistry.

The long-term vision for this work is to harness the capacity of proteins to self-assemble in response to a specific stimulus in order to create smart materials, responsive surfaces and nanodevices. The proposed research contributed to the science needs in topic 8.5: “—A particular area of interest is in the interface between nanostructures and biomolecules ... to generate advanced materials”. Down the track, we envisage that the knowledge gained in this program will inform the development of catalysts, scaffolds, surfaces and responsive materials with a wide range of applications, including those of military relevance, such as drug delivery, nanoelectronics, intelligent materials that respond to a specific stimulus, and self-healing materials.

## 6. Model systems chosen:

In selecting the two model proteins for study, we chose systems that satisfied the following criteria: a. an innate ability to form elaborate quaternary structures; b. ready access to appropriate constructs and in-house knowledge of how to express and purify the proteins, and appropriate variants; c. a common architecture (the toroid), enabling learnings from each objective to be readily tested for their generality in a second system; d. availability of thermophilic proteins to maximize outcomes for downstream applications.

**Lsm: a model capture system:** Lsm proteins self-assemble into a toroidal structure that binds to RNA, and has been selected as a model nanostructure with the ability to capture another molecule. Downstream, such components will be needed to develop sensing systems. The ring morphologies of several Lsm assemblies from diverse organisms have now been defined at atomic level, and range between 58-75 Å in diameter with central pores of 6-15 Å (Moll *et al.*, 2011). Regardless of specific amino acid sequence, each separate protomer within the Lsm ring assembly folds into a highly bent five-stranded-sheet module, generally capped by an N-terminal  $\alpha$ -helix. Strands 4 and 5 of each unit each lie at opposite edges of the module, so providing the key interaction points for adjacent Lsm subunits. Stacking of five to eight Lsm proteins in such a manner causes the formation of the toroid oligomer characteristic of all Lsm complexes (Fig. 1). RNA is known to engage at specific locations of the toroid, and the charged side chains critical to this molecular engagement are known.

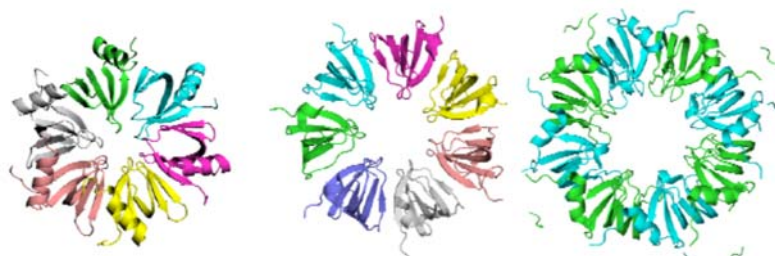


Figure 1: Examples of Lsm rings comprising 6, 7 and 8 protomer units. These distinct arrangements are naturally formed by Lsm proteins Hfq, MtLsm $\alpha$  and  $\gamma$ Lsm3, respectively.

**Peroxisredoxin (Prx) proteins: a model for environmentally triggered assembly:** The Prx proteins are an expansive protein family that have been well characterised in a biological framework (Karplus & Hall, 2007) and display an intriguing repertoire of quaternary structures (Barranco-Medina *et al.*, 2009). Excitingly, in the context of this project, interconversion between some of these architectures appears to be naturally controlled by environmental triggers, such as a redox switch (Cao *et al.*, 2005; Parsonage *et al.*, 2005). The biological relevance of the larger assemblies (Fig. 2) remains obscure, indeed they may only form *in vitro*; however, the propensity of these proteins to self-assemble by means of a redox trigger opens up huge potential for creating a switchable molecular device or responsive material.



Figure 2: Self assembly of the peroxiredoxins. From left to right: dimer, decamer, catenane, dodecahedron, nanotubule.

## **7. Summary of important results**

We have summarised achievements against each of the original objectives listed in the proposal.

### Year 1 objectives

#### **Obj Y1.1 Investigation of Lsm ring forms engineered with altered diameter and pores**

A comparison of the sequences and structures of known Lsm proteins (Moll *et al.*, 2011) led to predictions that the ring size of Lsm proteins might be altered by rational site-directed mutagenesis of the monomeric unit. Particular attention focused on the inter sub-unit beta 4/5 interface (Naidoo *et al.*, 2008). Using Lsm $\alpha$  as the parent protein, several variant proteins were designed, mutagenesis carried out, and protein expression trialled. As previously reported, most of the mutations did not change the size of the ring, suggesting that the interface is more resilient to mutation than first predicted. Repeated analysis of the mutations threw up two interesting observations: two mutations (L70A and I71A) were identified that significantly disrupted the oligomerisation, resulting in a monomeric species. Secondly, the mutation R65P consistently showed properties consistent with a hexamer rather than the native heptamer. These results were included in Akshita Wason's PhD thesis that is now complete. Work on this objective was completed, and provided material for crystallographic investigation in Year 3 (Obj. 3.4).

#### **Obj Y1.2 Physical characterisation of morphology and control of stacked forms of Lsm**

In addition to the results reported previously, further work in this area has been carried out and protein variants of particular interest are described below (see objectives Y1.3, Y2.1, Y2.2, Y2.6).

These results were included in PhD theses of Akshita Wason's and Francesca Manea. The only remaining work in this objective will be to complete publication of journal articles on the use of Lsm $\alpha$  and Lsm3 as protein tectons.

#### **Obj Y1.3 Generation of Cys and other derivative forms of Lsm rings**

As previously reported, careful examination of the crystal structure of Lsm $\alpha$  led to the design of mutant Lsm $\alpha$ N10CE61C, anticipating that this protein would form covalently bonded stacks. This was indeed the case, with stacks of typically 20 rings forming. Further work confirmed that the tubes could undergo reversible assembly according to the redox conditions in the protein's environment. This switchable assembly is a very exciting development and we are very close to submitting a manuscript to the ACS Journal '*Biomacromolecules*' based on the results in Akshita Wason's thesis.

#### **Obj Y1.4 Characterisation of assembly properties of hPrx 3 and hPrx3S78A with and without His-tag at a range of pHs**

This work was summarised previously. The manuscript describing this work has been published, based on results in the completed thesis of Amy Phillips, in the ACS Journal '*Biomacromolecules*' (see highlights).

#### **Obj Y1.5 Characterisation of B-peptide interaction with toroidal stacks of hPrx3 and Lsm (and mutants)**

In this objective, we explored the assembly properties of the B-type interface of hPrx3, using peptides as a probe. In particular, we explored the designed peptide IKHLSVN to mimic the properties of the B-type interface and to disrupt or stabilise larger assemblies of these proteins. Baseline studies of the peptide alone revealed a tendency to assemble into highly ordered ribbons and, further, has inspired a new class of tectons with the design drawn from examining the sequences of the interface of homodimeric proteins comprised of anti-parallel beta-sheets. This work was published in *Chemical Communications* and was previously reported.

Subsequent studies on the interaction of the peptide and Prx3 confirmed preliminary data and with optimization have yielded intriguing assemblies of stacked and laminated toroids, only in the presence of the peptide. This is a very exciting development, and work continues in collaboration with Dr Celine Valery (now at RMIT) to fully characterise the molecular mechanism of assembly and final structure of both the peptide ribbons and the peptide-protein assembly. This work is ongoing.

Excitingly, this peptide has been used as a component of a Bio-FET device in collaboration with other researchers in a related programme. This work has been published in '*Advanced Functional Materials*' (see highlights).

#### **Obj Y1.6 Characterisation of assembly properties of hPrx3 and appropriate mutants using tags, metal ions and redox conditions**

In order to gain greater control of the dimer-toroid assembly, Yewdall has engineered an obligate dimeric version of hPrx3 by introducing mutation S75E. SEC and SAXS analysis confirm a monodisperse population consistent with a dimeric species.

As previously reported, hPrx3S78AC47S, engineered by Phillips, was designed with reference to a recent X-ray crystal structure of a double toroid Prx structure (Saccocia *et al.*, 2012). This work was described in the *Biomacromolecules* paper.

Work continued across the team to examine the impact of the His-tag on the assembly process. The His-tag has been seen to not only stabilise the toroidal form of hPrx3, but also to encourage the formation of small stacks of toroids, which are not seen in TEM images of native samples. Juliet Gerrard's move to Auckland facilitated higher resolution imaging of these structures by cryo-EM, work that was published this year in '*Structure*' (see highlights).

#### Objectives for Year 2

#### **Obj Y2.1 Physical characterisation of morphology and control conditions for Lsm polyprotein polymers (Macquarie-funded PhD student).**

Samples of Lsm[2+3] and Lsm[4+1] polyproteins can now be routinely produced as rings containing 4 fused dimers. TEM micrographs of both polyprotein preparations clearly confirm that they comprise a ring-based morphology, as reported previously. Solution characterisation by SEC and SAXS indicated that Lsm[2+3] preparations form a mixture in which a single tetramer ring form predominates, yet Lsm[4+1] exclusively forms into double rings. Solution characterisation by SEC indicates that Lsm [4+1] and Lsm [2+3] preparations form a mixture in which a single tetramer predominates. Thus we have gained control over the supramolecular assembly process.



### **Obj Y2.2 Physical characterisation of morphology and control of stacked forms of Lsm (ARO-funded RA)**

This work was reported last year. Work from Objective Y2.1 expands our understanding of assembled forms of Lsm.

### **Obj Y2.3 Physical characterisation and exploration of solvent conditions for Lsm arrays (ARO-funded RA)**

This work was planned to use a self-assembling peptide to trigger a 2D ring array using Lsm polypeptide rings. This objective was not prioritised, to allow pursuit of fabrication of new and diverse tubule and caged Lsm forms and incorporation of Lsm into ordered arrays within a nanomaterial, in a separately funded programme (published in *Nanoscale*, see highlights and Figure 3).

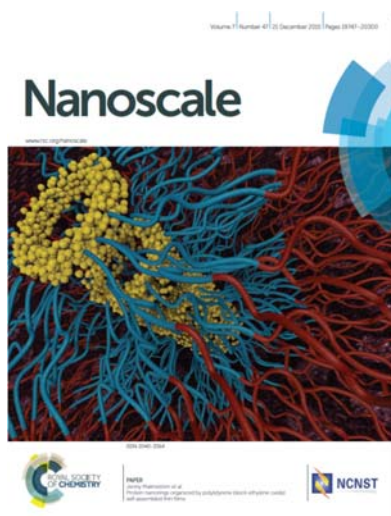


Figure 3: Artist's impression of Lsm proteins organised within a block co-polymer, as featured on the inside front cover of *Nanoscale*, November 2015.

### **Obj Y 2.4 Functionalisation and assembly of Prx derivatised with fluorophores (ARO-funded PhD)**

Unnatural amino acids have been successfully incorporated into the peroxiredoxin protein to selectively introduce clickable functional groups onto specific locations on the tecton. An *E. coli* expression system containing aminoacyl tRNA synthetase and tRNA that have been co-evolved to incorporate p-azidophenylalanine into amber codons (Chatterjee 2013; Chin 2002) has been successfully employed, after considerable optimisation efforts. Clickable fluorophore units that attach to azide groups have been successfully included as proof of principle that the system is working.

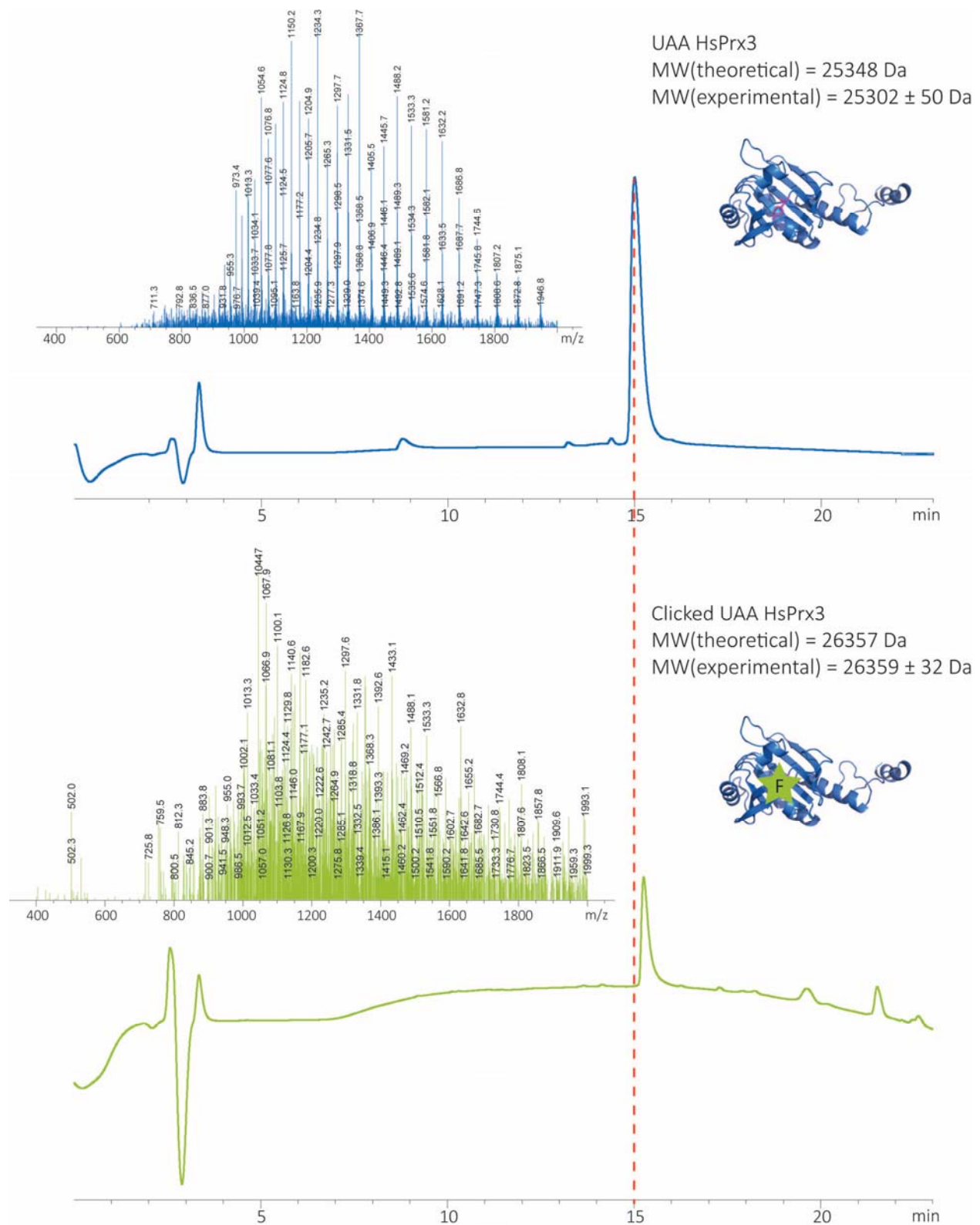


Figure 4: LC-MS data for unnatural amino acid (UAA) protein before and after click chemistry with DBCO-Cy5 fluorophore. The molecular mass is for monomeric protein, as seen in the cartoon representation of the crystal structure. Absorbance at 280 nm shows protein peak eluting from reversed-phase HPLC column, clicked protein having a slower retention time than the un-clicked protein.

## Obj Y2.5 Engineering hPrx3 toroids of increased pore size and analysing the influence on their propensity to assemble into stacks

Changing the pore size of both proteins has proved challenging. In the hPrx3 system, a protein was engineered that grafted onto the normally dodecameric protein a consensus sequence from a Prx2 and Prx1 protein that are known to be decameric. The new protein was soluble and folded, but preliminary studies shows the protein behaves as a dodecamer in solution. Crystals of the protein were obtained but only diffract to low resolution. This work was supplemented by control of toroidal stacking by tags, pH and peptides, since early results in this area were very exciting (see objectives Y1.4, 1.5 and 1.6 above).

## Obj Y2.6 Testing of appropriate toroidal structures in metal-templated assembly (MacDiarmid-funded PhD student)

As previously reported, exciting results were obtained by Wason, in collaboration with the Steel group, using metal ions to trigger the supramolecular assembly of Lsm $\alpha$  toroids. Ni<sup>2+</sup> ions proved particularly successful in creating reversible assembly of interesting cluster structures. This work has been expanded to include the Prx system.

Control of the length of hPrx3 stacks has also been obtained by a combinatorial approach in which the length of a histidine tag is varied and the assemblies formed at different pHs and different concentrations of metal ion. Some representative data are included in Figure 5, where the assemblies have been analysed by both TEM and analytical ultracentrifugation, as well as SEC.

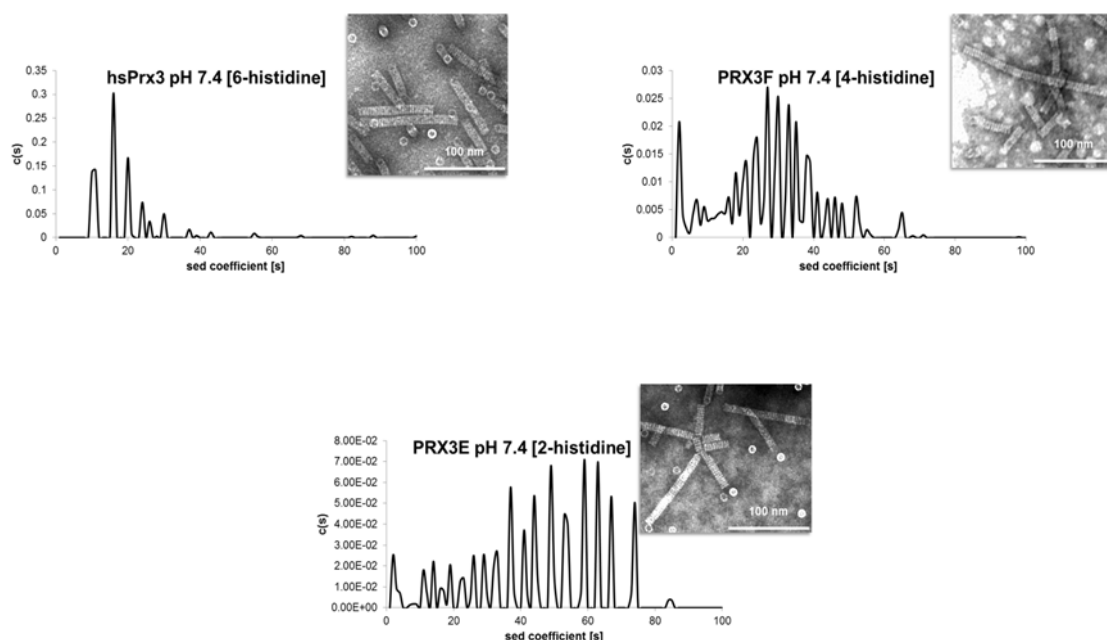


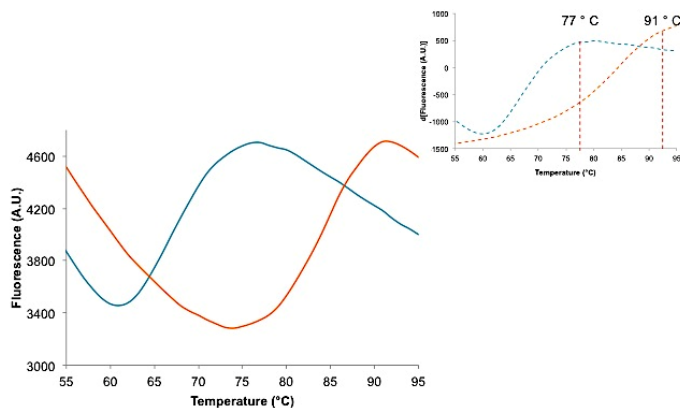
Figure 5: Controlling the size of hPrx3 stacked assemblies. Constructs with 6,4, or 2 histidines at the N-terminus form populations of different lengths in both solution, measured here by AUC and by TEM (inset) at different pHs.

## Year 3 Objectives

### Obj Y3.1 Generation of multifunctional Lsm rings utilising polyproteins (Macquarie-funded PhD student).

Lsm polyprotein ring tectons were probed for binding to RNA by both ITC (isothermal calorimetry) and DSF (differential scanning fluorimetry). Specific RNA responses for the Lsm polyprotein tectons is of interest, as a possible tuneable biomolecular recognition event for use in biosensor or diagnostic componentry. ITC results suggested tight binding of some of our Lsm tectons to the RNA sequence U<sub>2</sub>GCU<sub>4</sub>. DSF readout shows melting temperatures ( $T_m$ ) of tectons Lsm [4+1]<sub>4</sub> and Lsm [2+3]<sub>4</sub> to be 62° and 77° C, respectively (400 mM NaCl, pH 8.0). In the presence of U<sub>2</sub>GCU<sub>4</sub>, Lsm [2+3]<sub>4</sub> is significantly elevated in stability, with  $T_m$  increased to 91 ° C (see Figure), suggestive of strong and selective binding to this U-rich oligonucleotide. In contrast, tecton Lsm[4+1]<sub>4</sub> did not respond as markedly, with only a four degree change in  $T_m$ .

These data demonstrate that the specific componentry of the polyprotein tectons does impact the RNA-responsiveness of each Lsm ring, and therefore provides a tunable aspect to functionalisation of these materials. During 2015, an expanded repertoire of Lsm tectons was explored. Expression of the polyprotein Lsm[1+4] proved not to yield stable samples (unlike its symmetry relative Lsm[4+1]); however, recombinant Lsm[5+6] was found to be successful for production of a single ring tecton with new polyprotein componentry. Optimisation of solutions of this new material is underway at time of reporting.



**Figure 6:** DSF profile for *apo* Lsm[2+3]<sub>4</sub> (cyan) and Lsm[2+3]<sub>4</sub> with U<sub>2</sub>GCU<sub>4</sub> RNA (orange) (2 mg/mL) exposed to heating at 1 ° C/min with 200 x SYPRO Orange solution. Derivative trace is upper right.

### Obj Y3.2 Derivatisation of Lsm in array or tubule forms (ARO-funded RA)

Following the success of Lsm $\alpha$  cysteine constructs, cysteine residues were positioned at prominent stacking sites within Lsm polyproteins and Lsm3 to trigger higher-order structures. The new engineered forms differ markedly from previous Lsm constructs. Size-exclusion chromatography (SEC) revealed a distribution of sizes, including distinct larger molecular organisations.

For Lsm [4+1], a kinetically-stable population corresponding to ~450 kDa (or four interacting rings) can be discerned (Figure 6). Atomic force microscopy (AFM) and EM has revealed these to be homogenous ~120 Å Lsm tecton clusters with a visible pore (Figure 6). SEC runs under reducing conditions shows a loss of these high MW disulfide-linked polymers and regeneration of single rings (Figure 7).

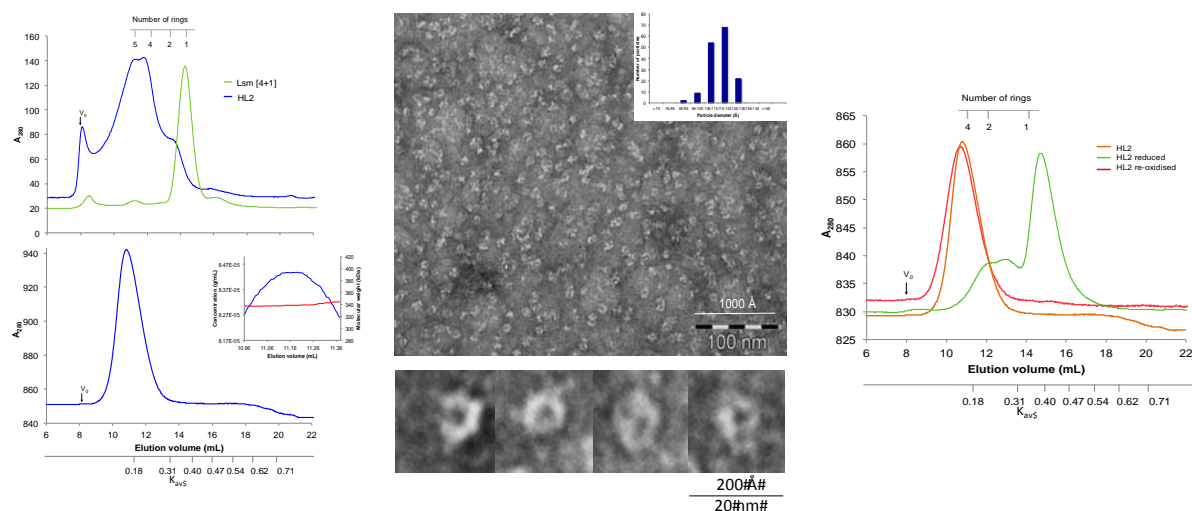


Figure 7: Engineering and characterisation of Lsm polypeptide Cys-linked cluster. SEC at pH 8.0 reveals a high MW organisation that can be individually isolated. Cluster morphology of approximately 12 nm is seen using EM (2.2 % uranyl acetate stain, 130 000x magnification). The reversibility of the system is illustrated with the addition of TCEP to reduce disulfide bonds, resulting in a large single ring population. Removal of the reducing agent leads to the generation of the protein cluster.

The hexa-His tag used for purification purposes was further utilised as a region to sequester metal. Several metals were titrated into pure Lsm [4+1] single ring tectons, with  $\text{Ni}^{2+}$ , similar to Lsm $\alpha$ , facilitating the formation of a higher-order organisation. EM has shown that the  $4^*\text{Ni-Lsm}[4+1]_4$  assembly resembles a cluster with resolved pores (rather than tubule formations), reminiscent of Lsm[4+1] complexes formed via Cys-linkages, (Figure 8).

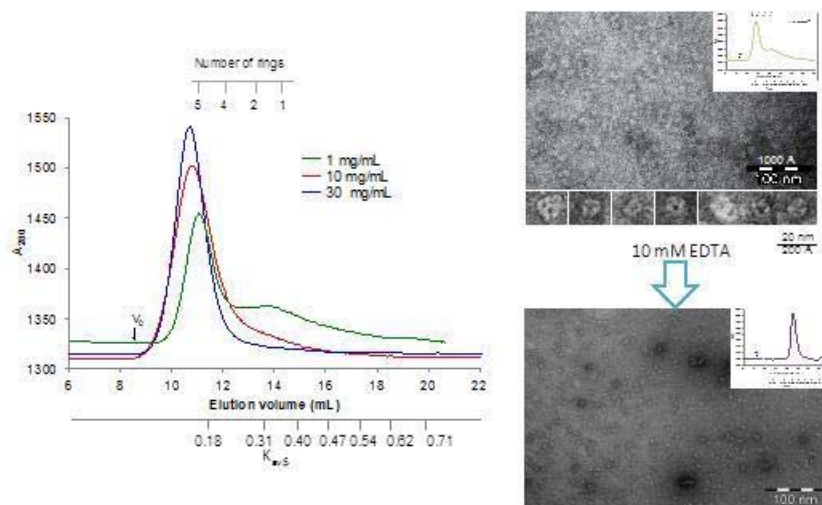


Figure 8: Physical properties of 4\*Ni-Lsm[4+1]<sub>4</sub>. SEC illustrates the concentration dependant relationship between protein and titrated metal. Clusters of 4\*Ni-Lsm[4+1]<sub>4</sub> visualised by EM, subsequently dissolve to single ring tectons with addition of EDTA.

The Lsm3<sub>8</sub> tecton (like Lsm $\alpha$ ) presents different coordination possibilities to the 4-fold symmetry polyproteins. When Lsm3<sub>8</sub> (containing His<sub>6</sub>-tags) is incubated with either Cu<sup>2+</sup> or Co<sup>2+</sup> at pH 8.0, distinct complexes of 190 kDa (i.e. comprising 2 ring tectons) are readily isolated. The 2Cu-Lsm3<sub>8</sub> complex is kinetically-stable to 15 mg/mL, and is dissociated into its discrete Lsm3<sub>8</sub> components by metal-chelating agent EDTA. When visualised by TEM (see Figure 9), 2Cu-Lsm3<sub>8</sub> particles (~10 nm in length) appear cylindrical, with visible pores. It is likely that this compact material exists as coaxially-stacked tecton pairs, and thus serve as a precursors to Lsm3-based tubules.

The T<sub>m</sub> for this Cu-containing material is relatively low, (50 °C), signifying that disassembly of this stacked Lsm cylinder might be triggered fairly readily by external stimuli. As expected for His-mediated conjugation, 2Cu-Lsm3<sub>8</sub> is pH-sensitive. At pH 6.0, a different organisation is seen in the presence of Cu, containing larger clusters consistent with 6Cu-Lsm3<sub>8</sub>. Under TEM, this can be seen as having a cage-like organisation, with pores visible.

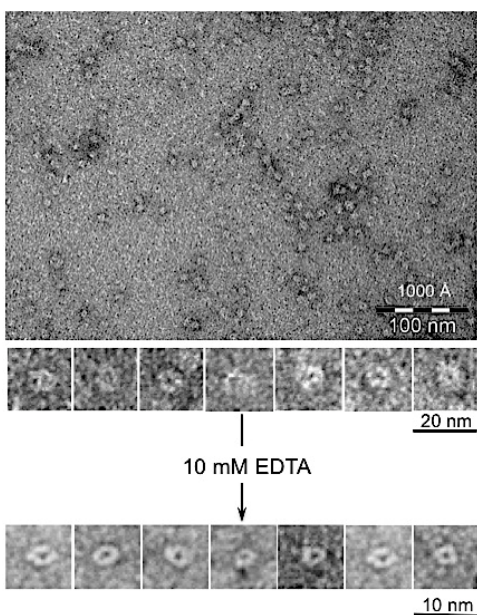


Figure 9: TEM visualization of 2Cu-Lsm<sub>38</sub> Electron micrograph at pH 8.0, negatively stained (uranyl acetate) at 130 000x. Individual selected particles display a pore. Selected ring particles observed following addition of EDTA are also shown.

### Obj Y3.3 Crystallography of Lsm forms (Macquarie-funded PhD student).

In order to efficiently construct with our tectons of synthetic Lsm rings, we first require information concerning the supramolecular architecture of our pure Lsm clusters in solution.

Crystallisation screens have been carried out on highly monodisperse samples of Lsm [4+1]<sub>4</sub>, a single ring tecton form, as well as larger Cys-linked and metal-conjugated clusters. Several conditions yielded crystals, and diffraction data on Lsm [4+1]<sub>4</sub> was collected at the Australian Synchrotron to 3.2 Å. However this was not of good enough resolution to allow phasing and structure determination.

Crystal conditions have since been optimised to grow larger crystals for all single and Cys-linked samples by varying precipitant conditions and utilising a microseeding approach. Very recently, the metal conjugated Lsm cluster underwent initial crystal screens and several conditions, giving microcrystals or crystalline matter, hold great promise for optimisation and eventual diffraction testing to gain a high resolution structure.



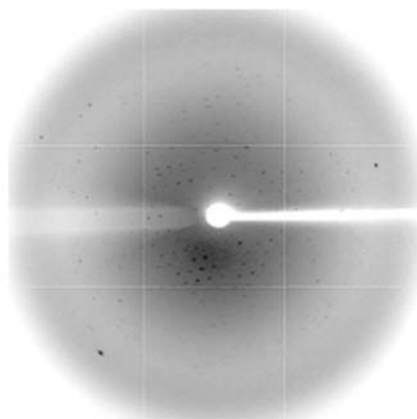
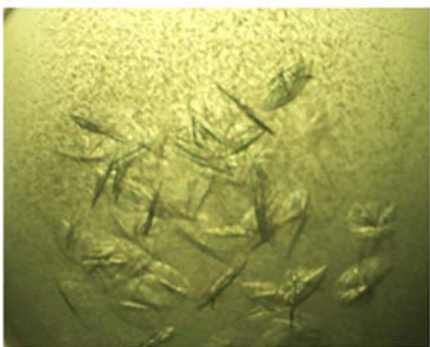
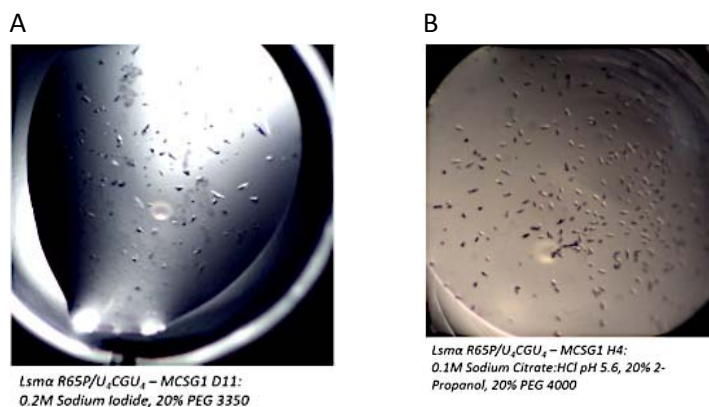


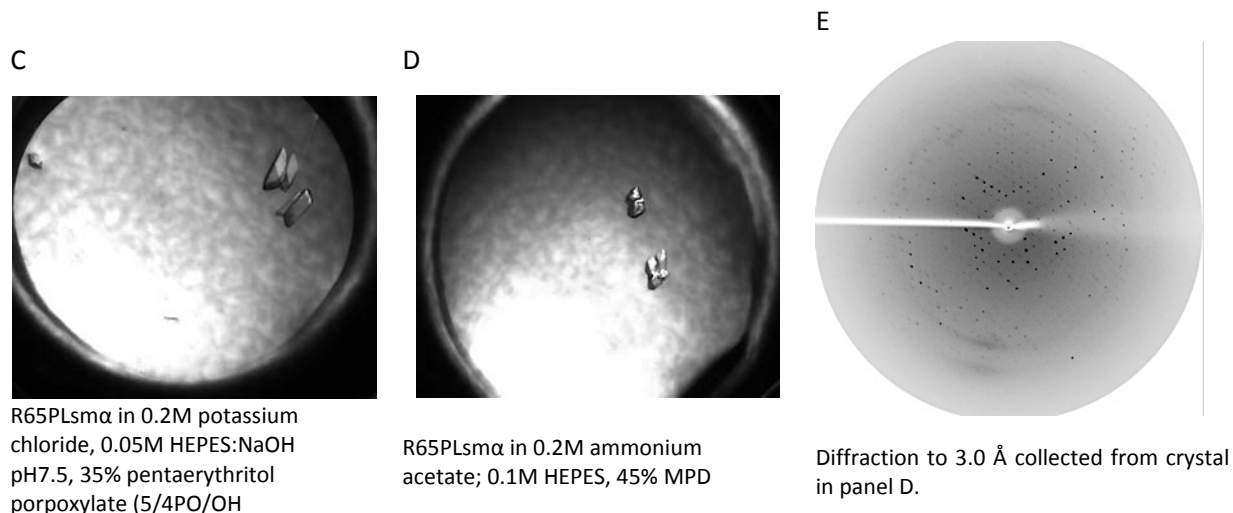
Figure 10: Crystals of Lsm [4+1]<sub>4</sub> (left) in 0.2 M magnesium acetate, 20 % PEG 3350. The recorded diffraction pattern at 3.2 Å is shown( right)

Following Wason's fabrication of an Lsm tecton tuned for a reduced internal pore, crystallographic investigation of the [R65P Lsm $\alpha$ ]<sub>6</sub> ring complex commenced at Macquarie in 2015. Highly pure material is readily produced at the Australian site, confirmed to be in a hexameric solution form, and microcrystals grown (from many conditions of sparse-matrix screens). Excitingly, these included both protein and protein/ U-rich RNA oligonucleotide mixtures (Fig. 9). Further optimisation of these crystals is underway at the time of writing.

One crystal of [R65P Lsm $\alpha$ ]<sub>6</sub> yielded 3 Å diffraction (space group P1, ), and a full dataset recorded at home source appears suitable for phasing and structure calculation. Such a structure will provide the basis of a detailed reporting of the fundamental molecular architecture of our re-tuned tecton, establishing design principles for future shaping of synthetic Lsm rings.







#### **Obj Y3.4 Functionalisation and assembly of Prx derivatised with antimicrobial peptides (ARO-funded PhD)**

Using the synthetic strategy described above (Objective Y2.4), we are now able to attach any moiety to the Prx scaffold which is amenable to click chemistry. This is a more sophisticated and generalised strategy than the one that we originally envisaged here, and was prioritised. The N-terminus has been derivitised with a variety of different peptide linkers, and this system can also be readily adapted to include an anti-microbial peptide if required.

#### **Obj Y3.4 Cloning, expression and characterisation of assembly properties of appropriate thermophilic Prx and Lsm, and mutants, building on the results of Years 1 and 2 (PhD, RA)**

Much of the work on Lsm proteins has employed a thermophilic protein source: Lsm $\alpha$ . A thermostable peroxiredoxin from *Aeropyrum pernix* (ApPrx) has also been sourced and behaves similarly to HsPrx3, in that under oxidising conditions it forms a dimer, and under reducing conditions it forms a decamer. ApPrx also stacks, as seen in TEM studies. With a melting temperature  $\sim 123$  °C, this thermostable protein will be studied in parallel with HsPrx3 in future studies and is a promising contender for thermostable tecton.

#### **Obj Y3.5 Testing of appropriate toroidal structures as surface coatings (MacDiarmid-funded PhD student)**

In collaboration with a MacDiarmid funded programme we have explored the use of His-tags as a means to order peroxiredoxins on a 2D surface. Exciting preliminary AFM data (Figure 12) suggests that this is a feasible way to create a nano-ordered surface.

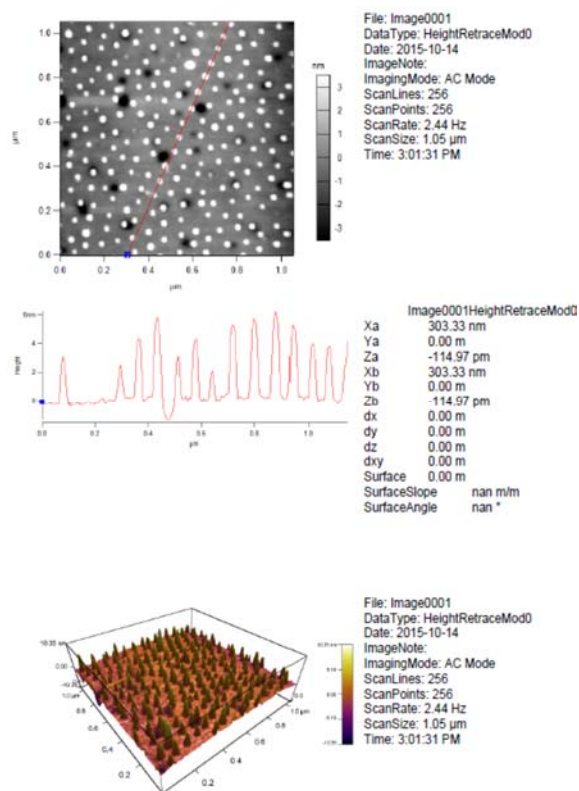


Figure 12: Preliminary AFM data showing hPrx3 ordered on a surface containing gold nanodots at defined spacing.

## 8. Conclusion

The objectives of the programme have been achieved. We have a created set of well-defined new tectons, some exciting supramolecular structures assembling from these tectons in response to specific chemical triggers, and some proof of principle studies of their utility in bionanotechnological applications.

## 9. Bibliography

Ballister, Lai, Zuckermann, Cheng & Mougous (2008), *In vitro* self-assembly from a simple protein of tailorable nanotubes building block. *Proc. Nat. Acad. Sci., USA* 105: 3733-3738.

Barranco-Medina, Lázaro & Dietz (2009), The oligomeric conformation of peroxiredoxins links redox state to function. *FEBS Letters* 583: 1809–1816.

Bath, Turberfield & Andrew (2007), DNA nanomachines. *Nature Nanotech.* 2: 275-284.

Behrens, Heyman, Maul, Essig, Steigerwald, Qunitilla, Wenzel, Burck, Dgany & Shoseyov (2009), Constrained synthesis and organization of catalytically active metal nanoparticles by self-assembled protein templates. *Adv. Mater.* 21: 3515-3519.

Chatterjee A, Sun SB, Furman JL, Xiao H, Schultz PG (2013) A Versatile Platform for Single- and Multiple-Unnatural Amino Acid Mutagenesis in *Escherichia coli*. *Biochemistry* 52: 1828-1837

Chin JW, Santoro SW, Martin AB, King DS, Wang L, Schultz PG (2002) Addition of p-azido-L-phenylalanine to the genetic code of *Escherichia coli*. *J Am Chem Soc* 124: 9026-9027

Cao, Roszak, Gourlay, Lindsay & Isaacs (2005), Bovine mitochondrial peroxiredoxin III forms a two- ring catenane. *Structure* 13: 1661–1664.

Collins, Harrop, Kornfeld, Dawes, Curmi & Mabbutt (2001), Crystal structure of a heptameric Sm-like protein complex from archaea: implications for the structure and evolution of snRNPs. *J. Mol. Biol.* 309: 915-923.

Collins, Cubbedu, Naidoo, Harrop, Kornfeld, Dawes, Curmi & Mabbutt (2003), Homomeric ring assemblies of eukaryotic Sm proteins have affinity for both RNA and DNA: Crystal structure of a heptameric complex of yeast SmF. *J. Biol. Chem.* 278: 17291-17298.

Das, Kozbial, Axelrod et al. (2009) Crystal structure of a novel-Sm-like protein of putative cyanophage origin at 2.60 Å resolution. *Proteins* 75: 296-307.

Hedde, Fujiwara, Yamadaki, Yoshii, Nishio, Addy, Yamashita, Tame (2007), Using the ring-shaped protein TRAp to capture and confine gold nanodots on a surface. *Small* 3: 1950-1956.

Karplus & Hall (2007), A structural survey of the peroxiredoxins. *Subcell. Biochem.* 44: 41-60.

Kozin & Svergun (2001), Automated matching of high- and low-resolution structural models. *J. Appl. Cryst.* 34: 33-41.

Ling, Reinhoudt & Huskens (2009), From supramolecular chemistry to nanotechnology: assembly of 3D nanostructures. *Pure Appl. Chem.* 81: 2157-2251.

Moll, Sobti, Mabbutt BC (2011), The Lsm proteins: ring architectures for RNA capture, in "RNA Processing". Intech ISBN: 978-953-307-332-3.

Naidoo, Harrop, Sobti, Haynes, Szymczyna, Williamson, Curmi, & Mabbutt (2008), Crystal structure of Lsm3 octamer from *Saccharomyces cerevisiae*: Implications for Lsm ring organisation and recruitment J. Mol. Biol. 377: 1357-1371.

Parsonage, Youngblood, Sarma, Wood, Karplus & Poole (2005), Analysis of the link between enzymatic activity and oligomeric state in AhpC, a bacterial peroxiredoxin. Biochemistry 44: 10583-10592.

Saalfrank, Maid & Scheurer (2008), Supramolecular coordination chemistry: the synergistic effect of serendipity and rational design. Angew. Chem. Int. Ed. 47: 8794-8824.

Saccoccia, Di Micco, Boumis, Brunori, Koutris, Miele, Morea, Sriratana, Williams, Bellelli & Angelucci (2012), Moonlighting by different stressors: crystal structure of the chaperone species of a 2-cys peroxiredoxin. Structure 20: 429-439.

Salgado, Radford, Tezcan (2010), Metal-directed protein self assembly, Acc. Chem. Res. 43, 661-672.

Sauter, Basquin & Suck (2003) Sm-like proteins in Eubacteria: the crystal structure of the Hfq protein from *Eschericia coli*. Nucl. Acids. Res. 31: 4091-4098.

Schalley, Lützen & Albrecht (2004), Approaching supramolecular functionality. Chem. Eur. J. 10: 1072-1080.

Steel (2005), Ligand design in multimetallic architectures: six lessons learned. Acc. Chem. Res. 38: 243-250.

Svergun, Barberato & Koch (1995), CRY SOL - a Program to evaluate X-ray solution scattering of biological macromolecules from atomic coordinates J. Appl. Cryst. , 28: 768-773.

Svergun (1999) <http://www.embl-hamburg.de/biosaxs/dammin.html>

Uhlenheuer, Petkau & Brunsveld (2010), Combining supramolecular chemistry with biology. Chem. Soc. Rev. 39: 2817-2826.

Ulijn & Woolfson (2010), Peptide and protein based materials in 2010: from design and structure to function and application. Chem. Soc. Rev. 39: 3349-3350.

Whitesides (2003), The —right size in nanobiotechnology, Nature Biotechnology 21:1161-1165  
Winterbourn & Hampton (2008), Thiol chemistry and specificity in redox signaling. Free Rad. Biol. & Med. 45: 549–561.

Zayed, Nouvel, Rauwald & Scherman (2010), Chemical complexity- supramolecular self-assembly of synthetic and biological building blocks in water. Chem. Soc. Rev. 39: 2806-2816.

Zheng, Birktoft, Chen, Wang, Sha, Constantinou, Ginell, Mao & Seeman (2009), From molecular to macroscopic via the rational design of a self-assembled 3D DNA crystal. Nature 461: 74-77.